CRISPR/Cas9 Mediated Mutagenesis in *Danio rerio*

Cassie Bullard
CRISPR/Cas9

• Acts as a rudimentary immune system in bacteria and archaea
• Creates double strand breaks
• Needs only single guide RNA
• Has recently been optimized for zebrafish
Design

Exon 1 → Target Sites → Target Sites → Exon 4

5' 3'

Designed Oligo

Overlap Sequence

Generic Oligo
Newly Synthesized Sequence

Overlap Sequence

Transcription

Guide RNA

Extension and Transcription
Floating Head

• Involved in notochord development
• If disrupted, embryos have just a head
• Known and well established phenotype
Wildtype
Floating Head Mutants
Where It’s Headed

- Ongoing project
- Can be used for individualized medicine
- Can possibly remove retroviruses (HIV)
- Gene therapy
Questions?